Cytomegalovirus viraemia in immunocompromised children in Cape Town

by

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List of abbreviations:

AUC	Area under the curve
BAL	Bronchoalveolar lavage
CI	Confidence interval
CMV	Cytomegalovirus
CMV VL	Cytomegalovirus viral load
EOD	End organ disease
ELISA	Enzyme-Linked Immunosorbant Assay
GCV	Ganciclovir
HAART	Highly Active Anti-Retroviral Therapy
HCMV	Human Cytomegalovirus
IHMF	International Herpes Management Forum
ICU	Intensive Care Unit
IQR	Inter-quartile range
IS	Induced sputum
IV	Intravenous
NPV	Negative Predictive Value
NPA	Nasopharyngeal Aspirate
PCP	*Pneumocystis Jerovecii* Pneumonia
PCR	Polymerase Chain Reaction
PMN	Polymorphonuclear Leukocytes
PMTCT	Prevention of Mother to Child Transmission
PPV	Positive predictive value

ROC	Receiver Operating Characteristics
OR	Odds ratio
SD	Standard deviation
UNAIDS	Joint United Nations Programme on HIV/AIDS

Chapter 1 Introduction and literature review

Human Cytomegalovirus (HCMV) first entered the realm of medical literature in 1904 when it was described as a large cell with intranuclear inclusions bodies. Found in the viscera and salivary glands of infants with what was then considered congenital syphilis, these cells which had an "owl's eye" appearance were initially thought to be protozoan-like. It was not until 1956, when cell culture techniques became available that HCMV was isolated from various laboratories(15;43;46). To this day, congenital HCMV infection is the most common viral cause of mental retardation and remains a public health priority worldwide.

HCMV is the fifth member of the family *Herpesviridae*. It belongs to the subfamily *betaherpesvirinae* and genus *Cytomegalovirus*. CMV shares the key characteristic of latent infection with its kin in the *herpesviridae* family, which allows CMV to persist in the host following infection. Like many other herpes viruses, primary CMV infection causes minimal disease in the infected host and is often subclinical.

Through complex cascades of viral gene expression, CMV is able to regulate its replication, evade the immune system and reactivate from its latent reservoirs from time to time. This biological property of HCMV can be observed in the clinical setting as intermittent viral shedding in the bodily fluids of infected but asymptomatic individuals. The persistent and insidious nature of CMV has important bearing on its epidemiology and transmission.

Epidemiology and pathogenesis of CMV

In non-industrialised countries where the standard of hygiene is poor, the prevalence of CMV infection is particularly high. Local seroprevalence data suggests more than 90% of adolescents in South Africa are infected with CMV(30). This in turn means that the majority of child bearing women may

shed CMV in the genital tract and beast milk. Swallowing of genital secretions and breast milk by the newborn during the perinatal period accounts for a large proportion of infection in this setting. In industrialised countries where the standard of hygiene is high, the adult seroprevalence of HCMV in the adult population is lower. Little more than half of pregnant women are infected with CMV(39). As a result, the common modes of transmission of CMV are between toddlers in crèches and playschools(2). This creates an interesting scenario in which the need for research into CMV is very different for developed and developing countries. In developed countries, many pregnant females are not immune to CMV and are at risk of acquiring primary CMV infection during pregnancy. This is associated with high risk for symptomatic congenital infection for the foetus(18). Therefore much focus in paediatric CMV research in the first world has largely been on prevention and diagnosis of congenital CMV infection. However, the research needs in developing countries are entirely different.

HCMV has a wide natural tissue tropism. This is evident as the virus can be cultured from secretions from various mucosal surfaces including saliva, urine, genital secretions and stool. During the time of reactivation, CMV inclusion bodies can be observed in the histological sections of various tissues, mostly glandular epithelium, endothelium, and white blood cells of myeloid lineage. Isolation of CMV from these sites does not necessarily indicate CMV end organ disease (EOD). Many tissues may be a reservoir of latent CMV infection from which CMV virions are produced on an ongoing or intermittent basis. The epithelial tissue of the salivary gland and kidney are the two main reservoirs of HCMV, but any cells capable of harbouring CMV may potentially be responsible for the persistence of the virus.

Most of the medical literature on Cytomegalovirus reports CMV disease in two groups of susceptible individuals. Congenital infection or CMV infection affecting the pregnant women and the foetus or newborn had been well described historically. In the modern era of medicine, CMV has also emerged

as a significant pathogen in the immunocompromised population. With the advent of transplantation medicine and the use of sophisticated immunosuppressive agents, CMV has become a formidable infection in both bone marrow and solid organ transplant recipients. CMV EOD's are commonly observed in such immunocompromised patients. The disease spectrum includes retinitis, central nervous system disease, oesophagitis, colitis, pneumonitis, and disseminated disease(28).

In Sub-Saharan Africa, the burden of disease is more commonly observed in another immunocompromised host, namely patients with advanced HIV infection. CMV end organ diseases in HIV infected adults, most commonly retinitis, CNS disease and gut disease, are AIDS defining illnesses that usually occur when the CD4 count is below 50 cells/μl. In the era of Highly Active Anti-Retroviral Therapy (HAART), CMV disease in HIV infected adults has largely been limited to those who have very limited access to HIV care. Although many patients seeking HIV care for the first time still present with very low CD4 counts, most CMV disease in sub-Saharan Africa is seen in HIV infected infants.

According to the latest Joint United Nations Programme on HIV/AIDS (UNAIDS) data, approximately 420,000 newborns were infected by HIV worldwide in 2007. Two million HIV infected children, representing 85% of all HIV infected children, are living in sub-Saharan Africa. They represent an important group of CMV disease that contributes to the majority of CMV disease burden in South Africa. In HIV infected infants and children, CMV disease may follow congenital infection or subsequent postnatal exposure. Due to impaired cell-mediated immunity, these children are unable to control CMV replication and may display more aggressive manifestations of local or systemic CMV disease. A recent Gambian study (29) confirms that transmission occurs in the first three months of life in most African children. Where infants are co-infected with HIV and CMV, the children are often profoundly immunosuppressed. HIV/CMV co-infection results, not only in

CMV disease in the various organ systems, but it accelerates HIV disease progression and predisposes the child to further opportunistic infections(31;38). Dually infected infants frequently have co-morbidities and the diagnosis of CMV EOD can be difficult and often requires numerous invasive procedures and a multitude of time-consuming laboratory tests to identify the cause.

Laboratory Tools for diagnosis of CMV

Methods of CMV identification have been constantly evolving for the last 50 years. The original viral culture method used to identify CMV is still very relevant today. Compared to the traditional culture of the past, modern CMV culture has been modified to reduce laboratory turnaround time. Instead of using a monolayer of human fibroblasts on a coverslip in sloped tube culture, specially adapted "Shell Vials" with round coverslips are used. Following inoculation of the clinical sample onto the human fibroblast containing Shell Vial, a centrifugation step is performed to disrupt the cell barrier and facilitate viral entry and infection of the cell monolayer. The culture is incubated at 37°C for 48 hours and stained with a CMV specific monoclonal antibody to identify viral antigen, in most cases product of an immediate early gene of HCMV. The process is shorted to two days without losing much sensitivity. To diagnose CMV infection in congenitally infected neonates, CMV Shell Vial culture on urine is still considered the gold standard today(5).

Serological assays detecting CMV IgG and IgM are also still widely used albeit in limited clinical contexts. Due to the indirect nature of antibody tests, serological assays for CMV have been restricted to checking immune status or detecting symptomatic primary infection in adults. CMV IgM is prone to false positive results. In addition, IgM assays may often be reactive during an episode of subclinical CMV reactivation, further limiting it use for predicting CMV disease. In developing countries where CMV infection and reactivation is common, CMV IgG and IgM have a very low positive predictive value for

primary CMV infection and CMV disease. In the paediatric setting where maternal antibody further complicates the issue, CMV Enzyme-Linked ImmunoSorbent Assays (ELISA's) play almost no role in diagnosis or management of CMV disease.

Histology is one of the oldest methods available for diagnosing CMV disease, but is still considered to be the most specific test for CMV disease today (35) because it is the only assay that can visualise the effect of HCMV at the site of the end organ. The characteristic "Owl's eye" appearance (or Cowdry type A intra-nuclear inclusions) is well recognised by histopathologists. There are two problems with using histology as the primary tool for the diagnosis of CMV disease. The first problem is the requirement of an invasive procedure to retrieve tissue samples. Procedures such as biopsy can only be performed by skilled staff and in an adequately equipped healthcare facility. Secondly the sensitivity of histology is especially dependent on the appropriate area with CMV disease being sampled and examination of an adequate number of sections. In an ill infant, invasive procedure to reliably obtain tissue from the diseased area is often difficult. Examination of multiple histological sections is time consuming and may be inefficient if the tissue sample is from a normal tissue.

During the search for better marker of systemic CMV disease, polymorphonuclear leukocytes (PMN) from individuals with CMV disease were found to contain CMV antigens. Although the pathogenesis of this phenomenon has never been elucidated, the technique of CMV antigenaemia was pioneered and has been used in the laboratory for screening for CMV activity in post transplant patients for many years. The principle of the assay consists of isolation of PMN and staining with a monoclonal antibody directed against the lower matrix protein of CMV, namely antigen pp65. This is a semi-quantitative assay and the result is expressed as number of pp65 positive cells per number of cells used to prepare the slide (usually 200,000 white blood cells). CMV antigenaemia has

been used to identify CMV disease in HIV infected patient with some success but its use in young infants in less than 6 months has been found to lack sensitivity. CMV antigenaemia has a few disadvantages, namely, the assay is labour intensive and requires sufficient number of white cells. Despite its relatively short turnaround time (5 hours of laboratory processing), the labour intensive nature makes antigenaemia assay tricky to perform when there are a large number of samples. In neutropenia or very young infants, obtaining sufficient white cells for the assay is often difficult. Furthermore, the fluorescent microscopy is subjected to the skill and experience of the microscopist.

Development of the Polymerase Chain Reaction (PCR) was one of the big breakthroughs in the field of molecular biology. PCR is able to amplify minute amounts of target nucleic acid and has since played an important role in diagnostic laboratories. In the field of CMV diagnostics, PCR is able to detect not only DNA from viable virus, but also viral DNA in body fluids or tissue where the infected cells have been lysed due to CMV replication. In many virology diagnostic laboratories around the world, the technology of PCR has made cell culture facility redundant. The exquisite sensitivity of PCR can often be a drawback as the presence of very low levels of viral DNA may not always be clinically significant. In recent years, further development of PCR has allowed the target to be quantified.

With the aid of quantitative PCR, virologists have been able to move one step closer to understanding the pathogenesis of HCMV by monitoring the replication dynamics of CMV in individuals susceptible to CMV disease. Observations from various prospective immunocompromised cohorts have revealed two interesting findings. Firstly CMV viraemia, or more correctly CMV DNAemia (presence of CMV DNA in the blood), is the main predictor of CMV disease in many immunocpmpromised cohorts(9;13;20). Secondly, serial monitoring of CMV viral load has shown that most individuals have a tolerance for a certain level of viraemia before developing CMV EOD. As the

CMV viral load increases, the likelihood of developing EOD remains low initially but when the tolerance threshold is exceeded, the probability of developing CMV disease increases exponentially. This threshold concept of CMV disease may be explained by the double tap and drain cartoon (Figure 1).

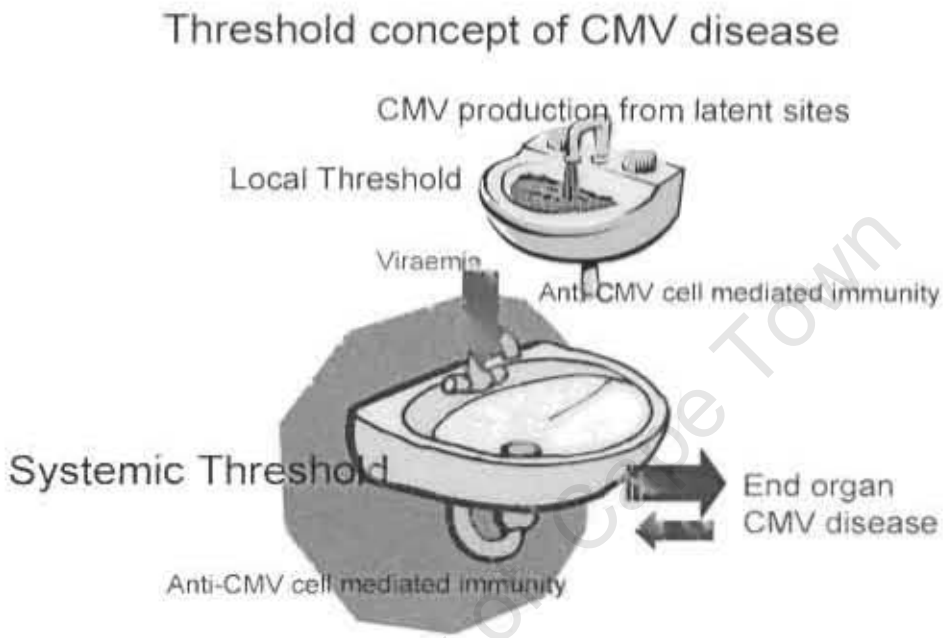

Figure 1 Interactions between CMV production, cell mediated immune response and CMV end organ disease. (Modified from Griffiths PD. Cytomegalovirus. In Zuckerman AJ, Banatvala JE et al ed. *Principles and Practices of Clinical Virology* 5th ed. John Wiley & Sons Ltd 2004)

The tap and small basin represents nominal CMV replication, insufficient to result in disease, from the latently infected cell population. This replication is kept in check by local cell mediated immune responses. When the local conditions favour excessive CMV replication, the virus spills into the systemic circulation (represented by the larger basin in the cartoon). The systemic immune response also controls the level of viraemia. When the host is sufficiently immunosuppressed, viral replication spills into the end organs leading to disease. The pathophysiology of CMV disease is likely to be multi-factorial and beyond the scope of this dissertation. The literature suggests

that direct cell lysis, disruption of integrity of the epithelium/endothelium, and/or immunopathology all play an important role in causing EOD when the threshold of viral replication is exceeded (23).

Real time quantitative PCR has been gaining popularity in pathology laboratories both locally and internationally. CMV viral load has made a quick transition from being a research tool to the gold standard assay in many virology laboratories. The technique combines the sensitivity of PCR and the specificity of fluorescent probes to provide a quantitative result with a relatively short turn around time. This makes real time PCR an attractive method for monitoring CMV activity in post transplant patients where rapid clinical decision-making and an early diagnosis is essential. Numerous studies examining the relationship between blood CMV DNA level and the risk of CMV disease showed a level of 5000-100,000 CMV DNA copies per ml of whole blood as the potential threshold for significant CMV disease(17). There is very little published data on the diagnosis of CMV disease in HIV infected children using real time PCR. Whether the data gathered from the adult post transplant setting can be translated to children, more specifically HIV infected children, remains uncertain.

CMV disease in paediatric settings covers a spectrum of different disease entities for which the diagnostic modalities are different. The latter half of this chapter will review some of the local issues regarding the diagnosis of CMV disease in various paediatric clinical scenarios and examine the value of diagnostic tools in each of the scenarios.

Congenital CMV infection

Firstly in infants that are less than three weeks of age, it would be useful to identify CMV infection as this constitutes the diagnosis of Congenital CMV infection and these children would be managed differently. The presence of

circulating maternal antibody renders the use of CMV IgG fairly limited. CMV IgM is known to produce false positive results and should be interpreted with caution. The diagnostic assay of choice for congenital CMV infection is the detection CMV in the urine of the newborn by culture or PCR. CMV viruria is not only the most sensitive marker of congenital infection, but also suggested by Arav-Boger and Pass(5) as the gold standards for which monitoring of treatment can be based. In the majority of symptomatic congenital CMV infections, the viral burden in the urine is high and as a result, culture of the virus is the preferred method as sensitivity is not usually an issue. In our scenario the majority of infants are brought to the clinician's attention only after the first three weeks with signs and symptoms that may suggest congenital infection eg, hepatosplenomegaly, microcephaly, jaundice, intracerebral calcification, failure to thrive and skin manifestations. In these situations, congenital infection can not be confirmed as the detection of CMV does not necessarily represent infection prior to birth. In order to manage these infants not only should CMV infection be confirmed but also CMV disease. Currently in many European countries foetal cord blood is collected by means of dried blood spot routinely performed after birth, and this contributes greatly to the monitoring and diagnosis of congenital infections, especially CMV(6). Collection of foetal blood at the time of birth is gaining popularity and may replace detection of CMV viruria as means of diagnosing CMV infection. This would be an interesting research area that warrants further study of viability. Currently there is no data available on the prevalence of congenital CMV in this country. The only published African study was done in Gambia by Kaye et al. This study showed that congenital infection was low (3,9%) However, this is a region of low HIV prevalence. The congenital infection may be higher in areas of high HIV prevalence as HIV infected women are more likely to have recurrent CMV infection and pass it onto the foetus.

Postnatally acquired CMV disease

Currently the diagnosis and management of postnatally acquired CMV disease in children depends on the nature of their clinical problem and the relative resources available to the attending paediatrician. For transplant recipients, the approach of prophylaxtic or pre-emptive treatment is used, while in HIV infected patients end organ disease is only sought when there is presence of symptoms. Antiviral therapy with ganciclovir (GCV) is instituted when end organ disease is confirmed in an HIV infected infant. The two approaches will be discussed separately.

The post transplant population has been well studied and a lot of the knowledge regarding CMV has been derived from prospective monitoring of these patients. The major difficulty with extrapolations from adult data is that children in South Africa are at a higher risk of developing CMV infection due to the high childhood CMV infection rate in this country. As a result, at Red Cross Children's Hospital all post transplant children at risk of CMV infection are put on prophylactic ganciclovir and are monitored for an increase in CMV activity. CMV pp65 antigenaemia assay is used in older children and children that are at a lower risk of CMV disease. CMVVL is used to monitor younger children and ill patients. According to the recommendation of International Herpes Management Forum (IHMF) the best type of sample for monitoring CMV DNAemia is whole blood as this is the most sensitive and reproducible sample type(40). The cut-off level of significant CMV activity in children remains undetermined as most of the studies involving children are small and the lack of standardisation makes comparison of data from various studies very difficult. Children with probable CMV disease are closely watched and the rate of increase of CMV viral load is the most accurate measure of CMV end organ disease in these children(40). Use of prophylactic ganciclovir and monitoring of CMV VL is costly. The use of diagnosis and management of CMV in this group of patients partly reflects the resources that are available to these patients.

At Red Cross Children's hospital, HIV infected infants and children are managed quite differently to the above approach. As serial monitoring of CMV is not possible with these children, a tool to diagnose CMV end organ disease at clinical presentation is required before appropriate pharmacological intervention can be instituted. Currently a combination of CMV DNAemia (by qualitative PCR) and detection of CMV at the end organ by PCR, histology or viral culture are used to diagnose CMV disease. The commonest presentation of CMV disease in this population is pneumonia in infants of 2-5 months. Due to the high mortality rate and poor outcome of this clinical presentation, HIV infected infants in ICU are routinely started on IV ganciclovir and this is only discontinued if the initial CMV DNAemia is absent. Infants that are less ill or do not meet the requirements for ICU admission, need to have CMV DNAemia (by qualitative PCR) as well as CMV cultured from the respiratory tract before ganciclovir is given. This approach in severely ill ICU patients may lead to overtreatment with ganciclovir as CMV DNAemia is quite a common occurrence in HIV infected infants. In the less ill non-ICU infants this approach may lead to both overtreatment and undertreatment. CMV is shed in the respiratory tract of most CMV infected infants and culturing of virus in these infants does not conclusively prove that CMV is the causative agent for the respiratory distress. There is no data comparing CMV culture and histology in this group of infants and it is very likely that both methods are fairly insensitive in diagnosing clinical CMV pneumonia. Similar problems are encountered in other CMV EOD such as GIT disease, CNS disease, retinitis and systemic CMV disease. On the whole, outcome of suppressive therapy after clinical presentation of CMV disease is poor compared to outcomes of prophylactic or pre-emptive therapy(48). Nonetheless a cost effective and practical approach to these HIV infected children currently used at Red Cross Children's hospital would save many lives.

Clinical management of CMV disease

International recommendations advise the treatment of paediatric CMV disease be conducted in two phases of pharmacological therapy: the induction phase followed by maintenance phase of therapy(36). The drug of choice for induction therapy is intravenous (IV) ganciclovir (5mg/kg/dose twice daily administered intravenously over 1-2 hours) for 14-21 days. This is followed by maintenance therapy which may be life long. Due to financial constraints, maintenance therapy is not currently offered for HIV infected children with diagnosed CMV EOD at Red Cross Children's Hospital. Currently 2 weeks of intravenous ganciclovir for a 4 kg infant will cost approximately R10,000, not including the cost of hospitalization and administration of the drug. The long-term use of IV or oral ganciclovir has not been considered feasible due to cost, practicality, poor bioavailability, toxicity and frequent dosing requirements. The risk of recurrent CMV disease following ARV induced immune reconstitution and discontinuation of ganciclovir treatment has not been studied although anecdotal reports from Red Cross Hospital of CMV related deaths occurring after commencement of HAART suggest that this may be a significant problem.

Research needs and purpose of this MMed

Currently in the field of paediatric CMV disease the following areas need to be addressed as a matter of urgency:

1. The diagnostic value of qualitative CMV PCR and quantitative CMV PCR on whole blood samples for distinguishing CMV disease from infection
2. The diagnostic value of Shell Vial culture on respiratory samples for CMV infection and CMV pneumonia.
3. Adequate period of ganciclovir maintenance therapy following induction therapy
4. An alternative to IV ganciclovir, as treatment of choice

5. Affordable monitoring tests for CMV activity (including possible congenital infection) in all HIV infected infants.

6. An evidence based, practical, and affordable approach to HIV infected infants with severe pneumonia

This study aims to address the current gap in our knowledge on the prevalence of CMV DNAemia in both asymptomatic HIV exposed infants and HIV infected infants with severe pneumonia. Without such data the meaning of this marker is unknown in this context. Further, there is a need to investigate an alternative good marker of CMV disease. Overwhelming CMV DNaemia has proven to be the only marker that independently predicts CMV disease across the various clinical scenarios. With the lack of better diagnostic modalities and serial monitoring of these patients, developing a reasonable cut-off of quantitative CMV PCR to predict CMV disease is crucial to improve the diagnosis of these disease entities.

Chapter 2 Material and methods

Study design and study population

This is a descriptive study designed to examine the prevalence and level of CMV DNAemia in whole blood samples from infants in Western Cape. Two groups of patients were examined:

1) Asymptomatic HIV exposed infants tested for HIV as part of South African national Prevention of Mother to Child Transmission (PMTCT) program.

2) Infants and young children admitted to Red Cross Children's Hospital with severe pneumonia.

For the asymptomatic infants, random samples were selected from blood samples sent to the diagnostic virology laboratory at Groote Schuur Hospital for HIV PCR testing between April 2007 and September 2007. Infants who were hospitalized, had pneumonia or had failure to thrive as part of clinical information were excluded from this study group. This group represents the HIV exposed but asymptomatic component of the study. In total 328 samples were selected and 45 samples were excluded due to incompatible clinical status. Mothers were counseled not to breastfeed their infants in this study groups and formula feed was supplied to those unable to afford it. Whether the infants were actually breastfed could not be determined from the information available.

The infants and children in the pneumonia group were taken from a prospective cohort recruited for a separate *Pneumocystis Jerovecii* Pneumonia (PCP) study based at Red Cross Hospital. The inclusion/exclusion criteria for the PCP study were as follows:

Inclusion criteria for PCP/CMV study

1. Severe Pneumonia: tachypnoea, recessions, O_2 saturations < 90% in room air
2. Suspected PCP: Severe pneumonia with one or more of the following :

- HIV suspected, HIV exposed or HIV infected children
- ICU admission, FiO2>60% or Head Box O2
- Malnourished or immunocompromised child e.g. chemotherapy, transplant recipient etc
- Bilateral pulmonary infiltrates on CXR
- Not responding to or deteriorating despite 48 hours of first line antibiotics and appropriate medical treatment
- Air leaks ie pneumothorax /pneumomediastinum (excluding empyema)

Exclusion criteria

1. No consent obtainable
2. On PCP treatment (Co-trimoxazole) > 7 days

At the time of this data analysis the study has yet to be completed and only the first 100 enrolled subjects have been included. This cohort represented the clinically ill group of the study. Five patients were further excluded because no blood samples were sent to the laboratory for CMV studies. In addition, for the pneumonia cohort, results of other investigations regarding CMV were also collected where testing had been done. They included Shell Vial culture on respiratory specimens and post mortem histology reports.

CMV qualitative and quantitative (CMV VL) PCR were performed to determine the prevalence and level of CMV DNAemia respectively for both study groups. Detailed methodologies of these assays as well as DNA extraction method are described below. To determine the positive predictive value of the CMV DNAemia for identifying infants with CMV EOD, a sub–group of infants from the severe pneumonia group were categorized as "CMV related pneumonia" on the basis that they fitted the following case definition:

- Clinical and radiological parameters compatible with interstitial pneumonia on admission

- CMV infection confirmed by qualitative PCR on whole blood
- Detection of CMV in the lower respiratory tract either by CMV Shell Vial culture on bronchoalveolar lavage (BAL)/Induced sputum (IS) samples or by histology on a post mortem lung specimen.

DNA extraction

Extraction of nucleic acid was performed on the automated Roche MagNA Pure instrument using the whole blood protocol (MagNA Pure LC DNA Isolation kit I Roche Diagnostics, GmbH Mannheim Germany) on 100 µl of whole blood. The choice of whole blood over plasma and peripheral blood mononuclear cells was based on both practicality and sensitivity. DNA was eluted into 100 µl of elution buffer from which both HIV and CMV PCR were performed. Negative controls were used with each extraction to monitor for possible contamination during the extraction process.

HIV PCR

Following DNA extraction, infants from both study groups were tested for HIV by means of PCR (Amplicor HIV-1 DNA test version 1.5, Roche Diagnostics, GmbH Mannheim Germany) as part of routine investigations. Other than the use of MagNA Pure DNA extraction instead of manual DNA extraction, the assay was performed according to procedure stipulated in the kit insert.

In house CMV PCR

Screening for CMV viraemia was performed on all samples by qualitative nested PCR targeting the Immediate Early (IE) region. The outer primers were design by Dr Heidi Smuts to increase the sensitivity and specificity of a single reaction PCR previously described by Ishigaki et al(25) For the outer reaction 10µl of extracted DNA was added to 40µl of mastermix consisting of 1.5mM $MgCl_2$, 200µM of each DNTP, 50mM KCl, 10mM Tris-HCl, 1.5U of Taq polymerase (Super-Therm, JMR Holdings, Kent United Kingdom) and 20 pmol of each outer primer. The outer primer sequences are as follows CMVs2 5'- GAG GCT ATT GTA GCC TAC ACT TTG G- 3' and CMVas2 5'- CTC TAT CTC AGA CAC TGG CTC AGA C- 3'. PCR was performed on an

Applied Biosystems Geneamp 9700 thermal cycler with the following cycling conditions: 94°C for 2 minutes, 35 cycles of 94°C for 20 sec, 58°C for 30 sec, 72°C for 45 sec and extension at 72°C for 7 minutes. 2µl of outer PCR product was transferred into the nested reaction with 48µl of mastermix containing 1.5mM $MgCl_2$, 200µM of each DNTP, 50mM KCl, 10mM Tris-HCl, 1.5U of Taq polymerase and 50 pmol of each inner primer. The sequences of inner primers were as follows: CMVs1 5'- CCA CCC GTG GTG CCA GCT CC – 3' and CMVas1 5'- CCC GCT CCT CCT GAG CAC CC – 3' (25). The cycling conditions for the nested reaction were 94°C for 2 minutes, 35 cycles of 94°C for 20 sec, 60°C for 30 sec, 72°C for 45 sec and extension at 72°C for 7 minutes. Detection of the PCR product was done by agarose gel electrophoresis and visualised under UV illumination with Ethidium Bromide. The PCR product size is 160 base pairs. Molecular marker VI (Roche Diagnostics, GmbH Mannheim Germany) was used to verify the size of the product. The analytic sensitivity of the nested PCR was not determined, but results from past external quality assurance panels suggest the nested CMV PCR has an analytical sensitivity of approximately 50 genome copies/ml sample input (data not shown).

Quantitative CMV PCR

Quantitative CMV PCR (Artus RealArt LC CMV PCR, Qiagen GmbH Hilden Germany), or CMV viral load (CMV VL), was performed on samples that were identified as positive by nested PCR in order to determine the level of viraemia. The assay was performed on the LightCycler 1.5 instrument (Roche Diagnostics, GmbH Mannheim Germany) according to the manufacturer's instructions. The analytical sensitivity of the quantitative assay is 541 genome copies/ml using our extraction procedure (namely 100µl sample extracted and eluted into 100µl). The quantitative result is dependent on input volume and the following formula is used to adjust quantitative result according to input volume.

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/µl) X elution volume (µl)}}{\text{Sample input volume (ml)}}$$

Negative controls and strict unidirectional workflow within the designated PCR areas were used to prevent contamination of all PCR performed. All tests were subjected to the routine quality assurance measures implemented by our ISO 15189 accredited laboratory during the course of the study.

Statistical analysis

Statistical analysis was performed using statistical software StataSE version 10. Shapiro-Wilk test for normality was performed. T test and Wilcoxon rank-sum test were performed to establish statistical significance between two normal and non-normal distributed sample groups respectively. Odds ratios with 95% CI were calculated using logistic regression. Receiver Operator Characteristics (ROC) curve analysis was performed to determine a CMV viral load cut-off that would best predict CMV pneumonia when compared to our case definition.

Ethics approval

Ethics approval was granted by the ethics review board of University of Cape Town/Groote Schuur Hospital and Red Cross hospital for both the asymptomatic and severe pneumonia components of the study.

Chapter 3 Results

CMV DNAemia in HIV-exposed asymptomatic infants:

Of 283 HIV exposed, asymptomatic infants (characteristics of this study group are listed in Table 1a), 191 were HIV PCR negative and 92 were HIV PCR positive. The prevalence of CMV DNAemia was significantly higher in HIV-infected infants ($p<0.001$) than in HIV-uninfected infants: 41% of the HIV infected and 17% of the HIV uninfected infants had CMV DNA detectable in their blood (OR 3.36, Std err 0.96, 95%CI 1.93-5.90) by nested in house PCR.

It was not possible to compare the prevalence of CMV DNAemia in HIV infected and uninfected infants under 2 months due to the small number of HIV infected infants in this age group, but 11% of HIV uninfected infants aged two months or less was found to have CMV DNAemia. This relatively low prevalence increased to around of 20% of HIV uninfected and 40% of HIV infected infants among infants older than two months. The proportion of infants with DNAemia remained fairly constant for the rest of the first year of life for both HIV positive and HIV negative infants (Figure 2).

CMV viral loads (CMV VL) were performed among asymptomatic infants with CMV DNAemia. (Table 1c) Despite the increased prevalence of CMV viraemia in HIV infected infants, the mean CMVVL in the HIV positive infants (Mean=3.59 ± 0.98 log genome/ml) was only marginally higher than that in HIV negative infants (Mean=3.31± 0.86 log genome/ml). The difference did not reach any statistical significance ($p=0.1$).

Table 1a. A summary description of the asymptomatic study group

characteristics of the asymptomatic PMTCT study group
• n = 283 • Median age 3.1 months (range 3 days – 12 months) • Clinically well. Hospitalised infants excluded from the study group • HIV exposed - participants of PMTCT program • Sourced from a variety of local clinics throughout Western Cape • Counseled to avoid breastfeeding as part of PMTCT and formula feed may be provided for some of the clinics • HIV PCR positive 92/283 (33%)

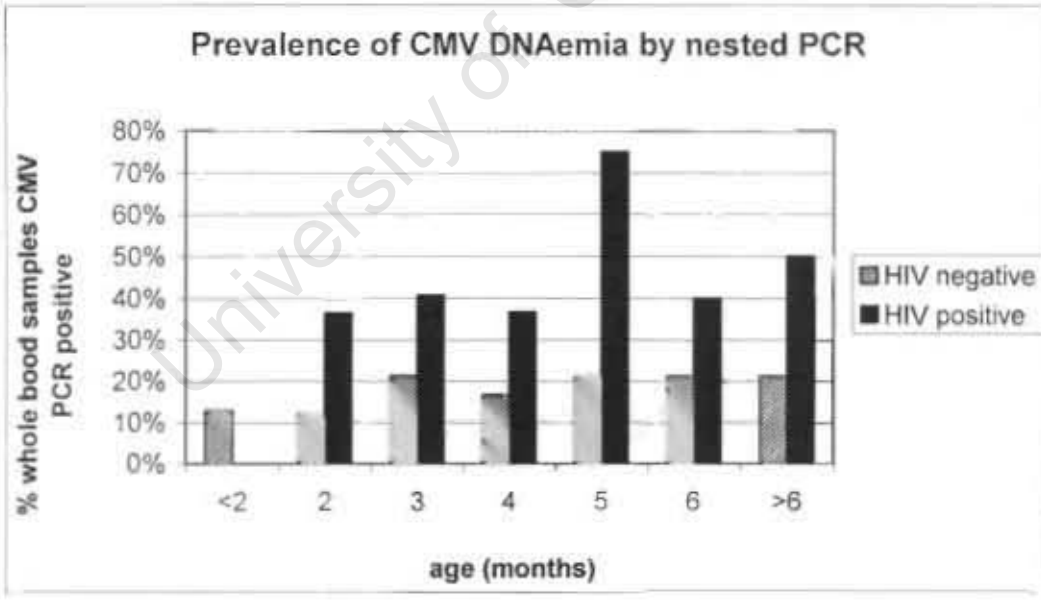

Figure 2: CMV Viraemia in HIV exposed asymptomatic infants by age. CMV DNA was detectible in the blood of asymptomatic infants from a very young age, rapidly reaching a steady rate of 20% for HIV negative infants and 40% for HIV positive infants. Age <2 months and 5 months categories of HIV positive infant may be anomalies due to a small sample size.

CMV DNAemia in infants with severe pneumonia:

Of the 95 infants (characteristics of this study group are listed in table 1b) in the severe pneumonia study group 30 were HIV PCR negative and 65 were HIV PCR positive. The median age (3.5 months) of children in this cohort was slightly older compared to that of the asymptomatic group (3.1 months), but the majority of patients were under 12 months of age. As was observed in the asymptomatic component of the study, CMV DNAemia was significantly more common in HIV infected infants (OR 4.18, Std err 1.95, 95%CI 1.68-10.44) (Table 1C). The proportion of children with CMV DNAemia in the HIV infected subset was 71%, almost twice that of the HIV uninfected at 37%.

The size of the group was too small to look at the prevalence of CMV DNAemia by age categories and HIV status. Instead, the proportion of infants with CMV DNAemia among age categories, irrespective of HIV status, was assessed in Figure 3. Among infants with pneumonia, 41% of age two months or less had detectable CMV DNA in their blood. The prevalence of CMV DNAemia increased to 89% in infants of two months and remained above 50% for the rest of the first year of life. Older children with severe pneumonia had a slightly diminished prevalence of CMV viraemia at 44%.

Breast milk is the commonest source of postnatal CMV infection. Using the breastfeeding history collected from children with severe pneumonia, we found breastfed children were at least 5 times more likely to be DNAemic for CMV (OR 5.39, Std error 2.86, 95% CI 1.9-15.2), but only marginally more likely to be HIV infected (OR 1.20, Std error 0.66, 95% CI 0.4-3.5). Table 3 illustrates the significant difference in the prevalence of CMV DNAemia between breastfed and non-breastfed children.

Table 1b. A summary description of the severe pneumonia study group. More than one possible causative agent was isolated from many of the patients in this study group.

characteristics of the severe pneumonia study group

• n = 95
• Median age 3.5 months (range 19 days – 98 months)
• Patients recruited from various wards in Red Cross Children's Hospital, include the intensive care unit.
• Clinically ill children with pneumonia of various aetiologies:
  o PJP 16/95
  o CMV 28/95
  o Tuberculosis 7/95
  o Bacterial pneumonia 26/95
  o Other respiratory viruses 25/95
• 36/96 (38%) participants of PMTCT program
• HIV PCR positive 65/95 (68%)

Table 1c. CMV viraemia rates by clinical status and HIV status

	Asymptomatic			Severe pneumonia		
	HIV uninfected (n=191)	HIV infected (n=92)	Total (n=283)	HIV uninfected (n=30)	HIV infected (n=65)	Total (n=95)
Median age ± IQR (month)	3 ±0.9	4±2.9	3.1±2.6	3.5±5.5	3.5±2	3.5±4
CMV PCR Pos (n, %)	33(17%)	38(41%)	71(25%)	11(37%)	46(71%)	57(60%)

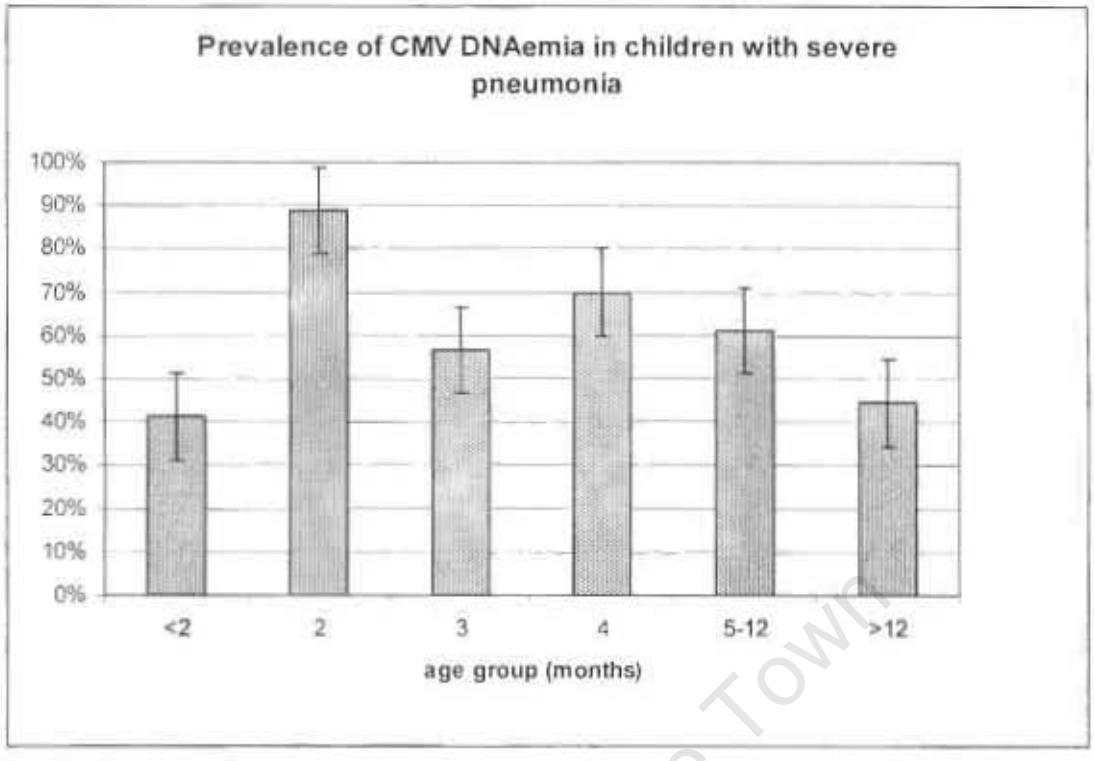

Figure 3 CMV DNAemia in children with severe pneumonia by age. CMV DNA was detected in the blood of infants who had severe pneumonia from very early in life. The number in each group is too small to further subdivide age group by HIV status.

Table 3 Comparison between breastfed and non-breastfed children with severe pneumonia. * p<0.01.

Breastfed	n	HIV pos	HIV %	CMV pos	CMV%
no	35	21	60%	14	40%*
yes	45	32	71%	34	76%*
unknown	15	11	73%	8	53%

CMV viral load in children with severe pneumonia

CMV viral loads were performed on all CMV viraemic infants. The average CMV VL in HIV infected infants with pneumonia at log 4.3 was significantly higher than in HIV-uninfected infants with pneumonia at log3.4 ($p=0.03$). It was also significantly higher than for asymptomatic HIV infected infants at log 3.6 ($p<0.01$), and asymptomatic un-infected infants at log 3.3 ($p<0.01$). (Table 1d, Figure 4)

Table 1d. CMV viral load of all CMV PCR positive subjects by clinical and HIV status. The CMV VL of asymptomatic infants (regardless of HIV status) and HIV uninfected infants with pneumonia were not significantly different. The CMV viraemia in HIV infected infants, however, was significantly higher than all three other categories ($p<0,05$).

	Asymptomatic CMV PCR positive			Severe Pneumonia CMV PCR positive		
	HIV uninfected (n=33)	HIV infected (n=38)	Total (n=71)	HIV uninfected (n=11)	HIV infected (n=46)	Total (n=57)
Mean CMVVL±SD (Log copies/ml)	3.3±0.9	3.6±1	3.5±0.9	3.4±0.9	4.3±1.2	4.1±1.2
Median CMVVL±IQR (Log copies/ml)	3.2±1.3	3.5±1	3.3±1.1	3±1.8	4.3±1.8	4.2±1.7

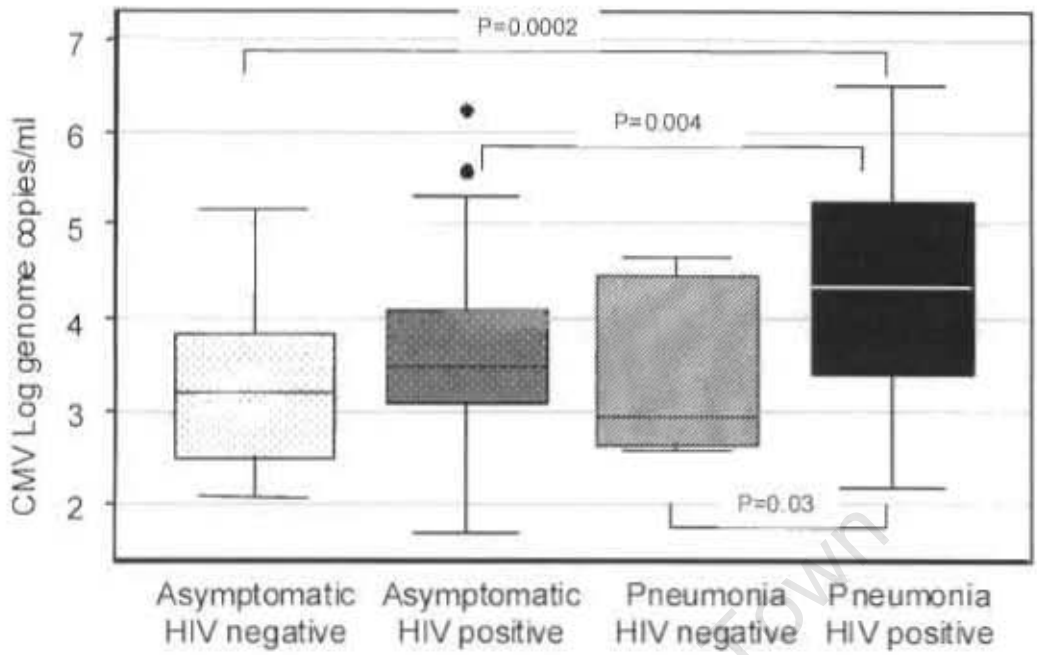

Figure 4. CMV VL of CMV PCR positive infants by clinical and HIV status. The CMV viral load in HIV negative infants with pneumonia was similar to that of the healthy infants. This could be considered the background level of CMV viraemia in the CMV infected infants of this age. In contrast, CMV viral loads were significantly higher in HIV infected infants with pneumonia. It is in this group that CMV is likely to play a role in the pathogenesis of HIV and/or CMV disease.

The infants in the severe pneumonia group were found to have a range of causes for their pneumonia, including other viral infections (25/95), bacterial causes (26/95) and PCP (16/95). A subset of 21 infants with pneumonia and CMV infection was selected according to our case definition to represent infants with probable CMV-related pneumonia. Clinical parameters of these patients are listed in table 4. The mean age of the infants with probable CMV associated pneumonia is 3.9 months. Of the 21 infants with probable CMV associated pneumonia: 17 were HIV exposed, 17 were HIV infected, 16 were known to be breastfed, 7 were co-infected with PCP and 4 had another respiratory virus cultured from the respiratory tract during the hospitalisation.

The mean CMV viral load of infants who fitted our case definition of CMV related pneumonia (4.58±0. 95 log copies/ml) was significantly higher than hospitalised children with non-CMV associated pneumonia (1.83± 2.09 log copies/ml, p<0,01) (Figure 5). When only CMV DNAemic infants were included for the same comparison, the difference between the two groups remains statistically significant (p=0.04, Figure 6). Our results also found that infants who fitted the case definition of CMV pneumonia were more likely to have been breastfed (OR 2.89 Std Err 1.67 95%CI 0.93-8.99) and HIV infection increase the likelihood of CMV related pneumonia although less significantly (OR 2.21, Std Err 1.21 95%CI 0.67-7.29). Among the severe pneumonia cohort, CMV is cultured from the NPA of16/21 patients with CMV associated pneumonia, significantly higher than those without pneumonia (10/74, p<0.01).

We examine the sensitivity and specificity of CMV culture for CMV infection, using the DNAemia as a gold standard. The two by two tables (Table 5 and Table 6) illustrate the performance of NPA and BAL/IS viral culture when compared to CMV DNAemia. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of viral culture from NPA were 51%, 91%, 88% and 59% respectively. The viral culture findings from BAL/IS were similar to that of NPA culture findings, namely 48%, 93%, 90% and 56% for sensitivity, specificity, PPV and NPV respectively.

To determine the CMV viral load cut-off value best able to discriminate between CMV pneumonia and non-CMV pneumonia, we performed ROC analysis (Figure 7). Assuming that all the cases analysed did have CMV pneumonia. We found that a CMV viral load cut-off value of 4.09 genome log/ml was able to identify the CMV disease status correctly in 81% of the cases with a sensitivity of 81% and specificity of 78%. The area under the curve is 0.8571 (95%CI 0.78-0.93).

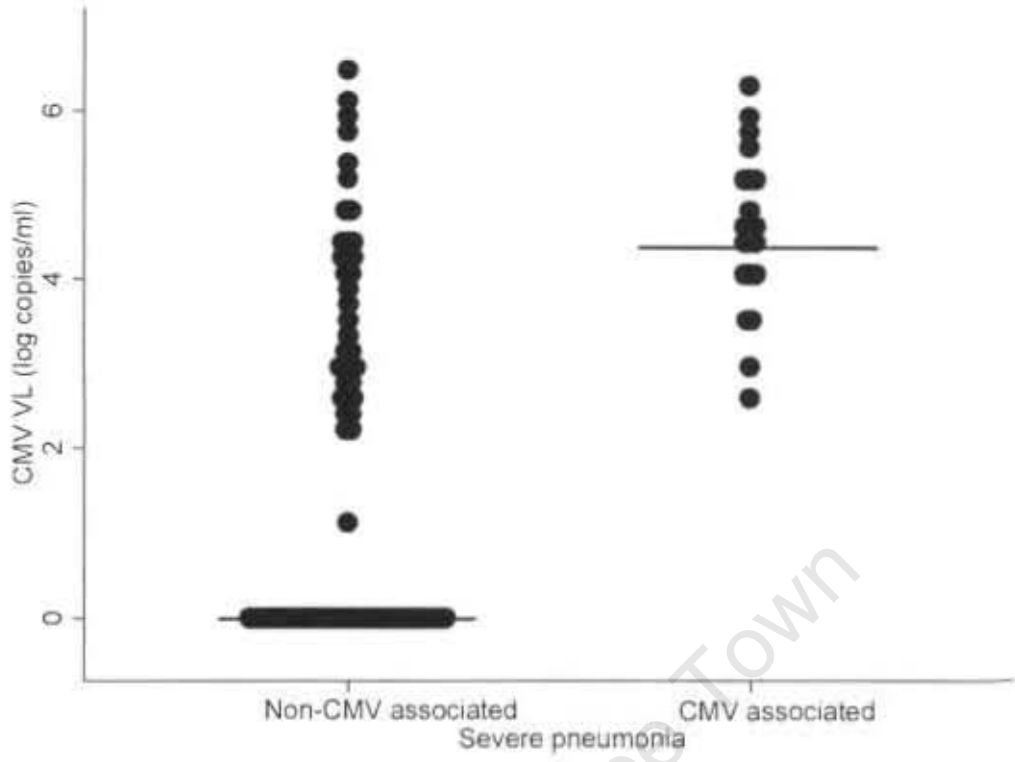

Figure 5. Scatter plot of CMV VL by CMV association. Each dot represents patients in the severe pneumonia group. CMV related pneumonia is defined by 1.Clinical and radiological parameters compatible with interstitial pneumonia on admission. 2.CMV infection confirmed by qualitative PCR on whole blood 3.Detection of CMV by either CMV Shell Vial culture from lower respiratory tract (i.e. BAL or Induced sputum) or histology on an autopsy lung specimen The median of each group are represented by the horizontal line.

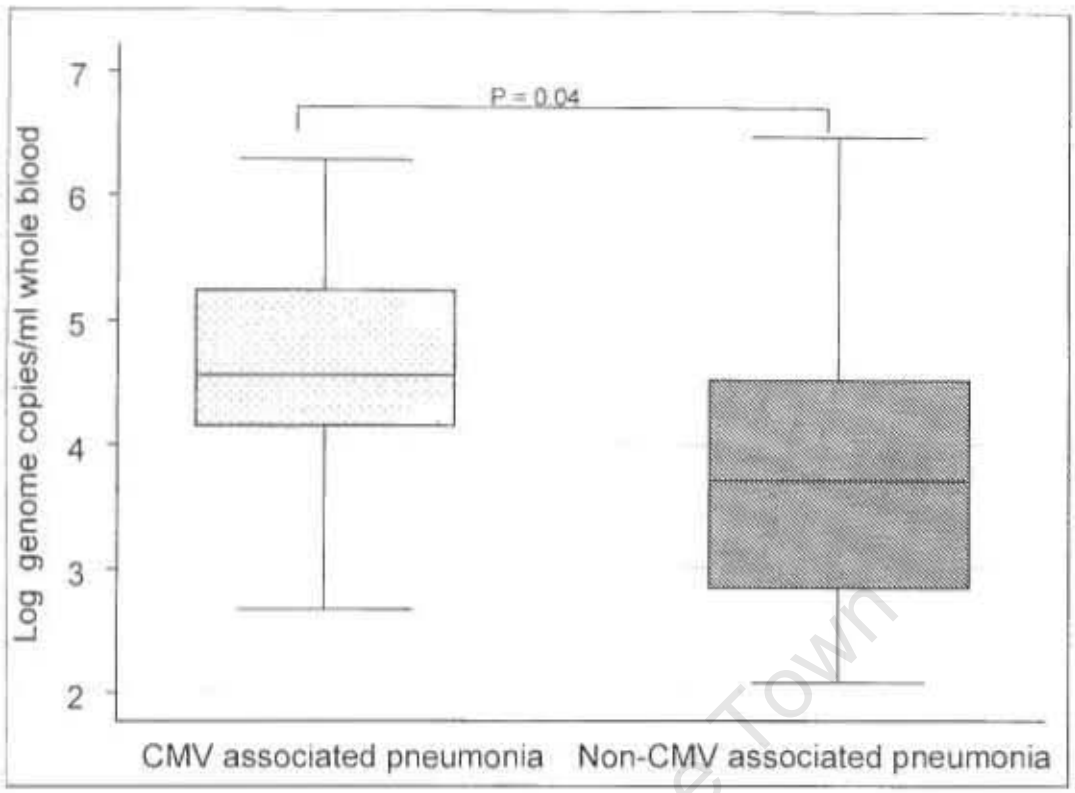

Figure 6. Box and whisker graph comparing the level of CMV viraemia among CMV DNAemic children with either CMV associated pneumonia or non-CMV associated pneumonia. 25-75 quartiles, the range and median of each group are represented by the box, whisker, and horizontal line respectively.

Table 5. Performance of viral culture from NPA. Of the 95 children from the severe pneumonia study, results for NPA culture and CMV PCR were both available for 76 children.

	DNaemia Positive	DNAemia Negative	total
NPA Culture Positive	22	3	25
NPA culture Negative	21	30	51
total	43	33	76

Table 6. Performance of culture from the lower respiratory tract (BAL and IS). Of the 95 children from the severe pneumonia study, results for culture from the lower respiratory tract and CMV PCR were both available for 69 children.

	DNaemia Positive	DNAemia Negative	total
BAL/IS Culture Positive	19	2	21
BAL/IS culture Negative	21	27	48
total	40	29	69

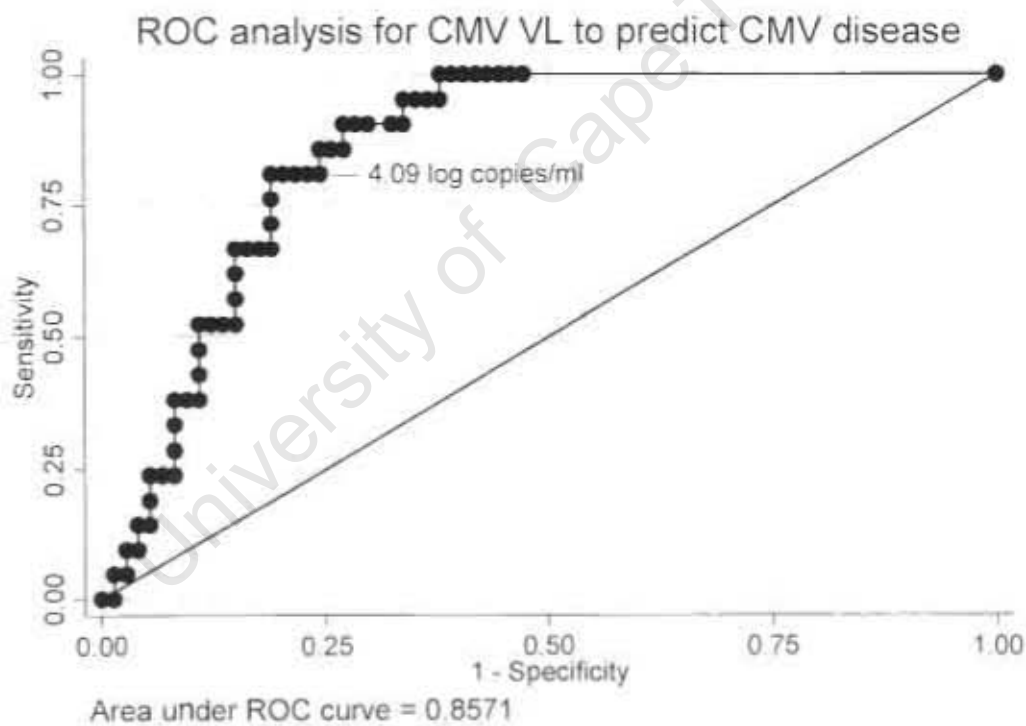

Figure 7. The ROC analysis graph was used to asses the utility of CMV VL in predicting CMV disease. A cut-off of 4.09 log copies/ml was able to best separate the infants with probable CMV pneumonia from those without.

Table 4: Table of clinical info of CMV pneumonia children

Age (months)	sex	HIV	Breastfed	CD4 count	HIV VL log	RR	SaO2	PJP	CMV culture		Other Viral culture	CMVVL	CMVVL log
									IS/BAL	NPA			
2.70	f	Pos	Unknown	15	ND	50	61	n	pos	neg	neg	177350	5.25
4.60	f	Pos	Unknown	76	>6.7	50	76	n	pos	neg	Parainfluenza 3	24255	4.38
3.80	f	Pos	No	40	ND	80	84	n	pos	pos	neg	967900	5.99
2.17	m	Pos	Yes	291	>6.7	80	66	n	pos	ND	neg	504220	5.70
2.80	f	Pos	Yes	629	>6.7	50	79	y	pos	pos	neg	14298	4.16
1.23	m	Pos	Yes	2793	>6.7	50		n	pos	pos	neg	51200	4.71
2.23	f	Pos	Unknown	135	5.99	60	90	y	pos	pos	neg	14165	4.15
2.70	f	Pos	Yes	488	>6.7	93	82	n	pos	pos	neg	2680	3.43
3.20	m	Pos	Yes	ND	6.48	68		n	pos	pos	RSV	152800	5.18
5.37	m	Neg	Yes	ND	ND	62	81	y	pos	pos	neg	42990	4.63

Age (months)	sex	HIV	Breastfed	CD4 count	HIV VL log	RR	SaO2	PJP	CMV culture		Other Viral culture	CMVVL	CMVVL log
									IS/BAL	NPA			
3.63	f	Pos	Yes	296	>6.7	40	89	y	pos	pos	neg	176100	5.25
2.97	f	Pos	Yes	157	ND	100	67	y	pos	pos	neg	27800	4.44
1.93	f	Pos	Yes	ND	ND	90	83	y	ND*	neg	neg	365850	5.56
3.27	f	Pos	Yes	526	ND	75	60	n	pos	pos	RSV	3281	3.52
3.33	f	Pos	Yes	934	>6.7	60	56	n	pos	pos	neg	1959000	6.29
2.97	f	Neg	Yes	ND	ND	60	89	n	pos	pos	neg	470	2.67
6.80	m	Pos	Yes	268	ND	50	86	n	pos	pos	Parainfluenza 2	27015	4.43
3.80	f	Neg	Yes	ND	ND	70		n	pos	pos	neg	36500	4.56
10.03	m	Pos	Yes	911	ND	64	88	n	pos	pos	neg	761	2.88
6.50	f	Neg	Yes	ND	ND	50		n	pos	pos	neg	68100	4.83
6.80	f	Pos	No	309	>6.7	70	77	y	Neg*	neg	neg	12340	4.09

IS – induced sputum

NPA – Nasopharyngeal aspirate

BAL – Broncho-alveolar lavage

ND – Not done

* CMV pneumonia confirmed by post mortem histology

Chapter 4 Discussion

CMV DNAemia in asymptomatic children

Although clinical and laboratory observations suggest that CMV infection in Africa occurs at a very early age, it is very difficult to establish the exact epidemiology and prevalence of CMV infection in this early age group. The main tool used to determine seroprevalence, namely CMV IgG, cannot be used in young infants due to the ubiquitous presence of maternal antibody. In order to examine the age at which most of African children become infected with CMV, direct techniques such as CMV viral culture or CMV PCR will need to be performed on infants serially. Thus only a prospective study capturing the vertical CMV transmission in mother-child pairs, followed closely throughout the first few months of life, would be able to demonstrate the prevalence of congenital CMV and rate of acquisition of postnatal CMV infection. This important data of CMV epidemiology was lacking for African infants until the recent Gambian study conducted by Kaye et al. A cohort of mother-newborn pairs was follow up for 12 months to look for the source and timing of childhood CMV infection in Gambia. Their results showed approximately 75% of infants were infected by 6 months of age and 85% of infants were infected by one year. The cumulative prevalence of CMV over time showed that the majority of infants were infected with CMV between 8 and 16 weeks. The incubation period of CMV is 4-8 weeks and if we extrapolate the Gambian data, the time of infection would be in the first two months of life. The implication of this finding confirms the suspicion that perinatal infection of CMV, especially through breastfeeding, is an important route of transmission in Africa. According to UNAIDS statistics, the HIV prevalence in adults in Gambia is around 2.7% and HIV infected infants would have comprised a relatively small proportion of their study cohort. In a high HIV prevalence area like South Africa, the number of CMV infected infants may be higher as HIV infected mothers are likely to shed more CMV than healthy women. Thus more local research is required to determine the epidemiology of infantile CMV infection in our setting.

In this study we examined the prevalence of CMV viraemia in HIV exposed well infants in Western Cape where the HIV prevalence among women attending antenatal clinics is 15.1% (the 2006 South African National Antenatal survey by Department of Health). The infants that were selected in this component of the study were all from the local clinics where PMTCT was offered. This may represent a potential sampling bias as PMTCT was offered in areas which have better access to health care. This sampling method may not accurately reflect the findings in rural parts of South Africa.

All infants were HIV exposed and had had their blood sampled for the purpose of determining their HIV status. Our results showed that overall 25% of HIV exposed asymptomatic infants had detectable CMV DNA in their peripheral blood. CMV viraemia was present at a very early age and was much more common in HIV infected infants (41%) compared to HIV uninfected infants (17%). The high prevalence of CMV viraemia observed at the early age in our study population is in keeping with the recent finding by Kaye *et al* which indicated that natural CMV infection frequently occurs in early infancy in Africa. Classically the most significant route of transmission of CMV for the African newborn is through breastfeeding. In our asymptomatic study group, mothers of the study subjects were given formula feeds and advised not to breastfeed as part of the PMTCT program. Despite this, our results indicate that more than 10% of two month old HIV exposed infants had CMV viraemia. The CMV viraemia rate remained fairly constant at around 20% up to the age of 12 months. This early DNAemia up to 3 months suggests that infection occurred during the early neonatal period, most likely during the childbirth process or subsequent close contact although the possibility that some may have acquired inection via breastfeeding cannot be excluded. Furthermore, detection of CMV in the urine is widely considered to be the most sensitive technique for diagnosing CMV infection as DNAemia is likely to be transient in most CMV infections. The DNAemia figures that we collected are likely to be an underestimate of the actual CMV prevalence in this group. Unfortunately due to the nature of

the study we were unable to examine the utility of DNAemia as a marker of CMV infection.

In a study by Kovacs and colleagues(31), HIV infected infants, despite having similar CMV infection rates at birth, were significantly more likely to be infected by CMV at six months (as determined by CMV culture in the urine). This conclusion is supported by our DNAemia data where HIV infected infants were more than twice as likely to have CMV DNAemia than HIV uninfected infants (Table 1a). A few factors might account for the high prevalence of CMV in HIV-infected infants. Firstly, the normal cell mediated immunity required to control CMV replication may be impaired in the HIV positive infants and as a result they may take longer to clear the CMV viraemia. Collectively this would influence the prevalence of viraemia. Secondly, the HIV positive infants represent the group where PMTCT has failed. The failure of PMTCT may be attributed to higher maternal HIV viral load, intermittent breastfeeding due to poor access to formula among other factors that could increase the risk of early CMV acquisition. Thirdly this may be a result of an increased incidence of congenital CMV infection which presents with persistent viraemia in the ensuing months following birth although whether maternal HIV infection increases congenital CMV infection remains controversial(16;37).

Based on our findings, a practical implication is that the role of CMV PCR needs to be re-examined in the current clinical setting. The high prevalence of CMV DNAemia in an apparently well cohort indicates that this very sensitive assay can detect presence of CMV that has not resulted in clinical disease. CMV PCR is currently being used as a guide to manage infants with suspected CMV disease at Red Cross Hospital but it has a very low positive predictive value for CMV disease. While its negative predictive value is good, a positive result needs to be interpreted in combination with other clinical or laboratory parameters before CMV EOD can be diagnosed.

In CMV viraemic infants, the level of CMV DNAemia was further defined by quantitative CMV PCR. Comparing the level of DNAemia between the HIV infected and HIV uninfected asymptomatic infants, the mean CMV viral loads

were similar, 3.59 log copies/ml for the HIV infected and 3.31log copies/ml for the HIV uninfected. Predictably, the difference did not reach any statistical significance. As the level of viraemia is the best predictor of CMV disease, we expected infants without EOD to have similarly low levels of CMV viral load. The marginally higher viral load in HIV infected infants may be due to poorer immune control of CMV in the HIV infected infants, but the effect of the small study sample size may limit further interpretation of this comparison. Nonetheless, the CMV viral load in our asymptomatic group is a novel finding and would be useful in guiding the interpretation of viraemia level found in symptomatic infants.

CMV DNAemia in children with severe pneumonia

Most healthy infants who acquire CMV infection in the first year of life cope well with the infection and CMV disease in healthy immunocompetent infants is very uncommon. Infants who are immunocompromised due to prematurity, congenital immunodeficiencies or HIV infection are more likely to develop symptomatic infections. They can either manifest as a glandular fever like systemic illness or end organ disease such as retinitis, pneumonia, colitis or CNS disease.

When examining CMV prevalence data in infants with severe pneumonia, two distinct trends emerged. Firstly the CMV DNAemia was very common in the first year of life: 41% of infants under the age of 2 months old were viraemic for HCMV, increasing to 89% by two months, and dropping to less than 50% by a year. This prevalence trend is consistent with the age at which infants present with CMV disease to Red Cross Children's Hospital, namely, in the 2 to 5 months age group. Primary CMV infection in African infant occurs commonly in the first two months of life. In HIV/CMV co-infected infants CMV pneumonia at 2-5 months of age may be a manifestation of persistent primary CMV infection. CMV related pneumonia has a high mortality in this age group and the lower prevalence seen in older children may reflect either infants that were infected later during infancy or a group of perinatally infected infants that had better control of CMV replication. A

prospective study is clearly required to investigate the relationship between primary CMV infection and CMV EOD. This may shed light on the reason why infants between 2-5 months are particularly susceptible to CMV disease.

Secondly, as observed in the asymptomatic component of the study, HIV positive infants were more likely to have CMV viraemia. The overall high prevalence of CMV DNaemia (71%) in these ill infants may reflect the higher prevalence of HIV in this group. Compared to the asymptomatic groups (HIV prevalence 33%), the HIV prevalence of pneumonic children were twice as high (68%).

Interestingly, the pneumonic infants were more than twice as likely to have CMV viraemia than healthy infants, regardless of their HIV status (37% vs 17% in HIV negative infants, OR 2.77 [95%CI 1.21-6.37] and 71% vs 41% in HIV infected infants, OR 3.44 [95%CI 1.75-6.77]). Studies have shown that in immunocompromised individuals, CMV infection is a contributor of illness and disease progression(1;11;27;42). Williams and colleagues found, among infants with vertically acquired HIV, CMV and PCP co-infection was associated with a worse prognosis than PCP infection alone(52). Frenkel et al. studied 38 HIV exposed infants prospectively and their findings suggested that 1) CMV infection plays an important role in HIV progression and 2) symptomatic CMV infection is associated with increased morbidity and mortality of these children(19). CMV is well known to have a detrimental effect in transplant patients (44). More recently, in a study by Limaye et al it was found that CMV reactivation plays an adverse role in critically ill immunocompetent patients as well (33). Further randomized control study looking at ganciclovir prophylaxis vs placebo in the critically ill immunocompetent patients are in the pipeline. Our data support the hypotheses that acute illness in general can result in reactivation of CMV. The difference in the proportion of patients with DNAemia between the HIV status-matched groups, 20% for HIV uninfected and 30% for HIV infected children, could represent the proportion of individuals with reactivation of

CMV during an acute illness. The role of ganciclovir prophylaxis should be similarly studied in critically ill immunocompromised infants.

The influence of breastfeeding on prevalence of CMV DNAemia in pneumonia cases was examined. Our data confirmed that there was a higher prevalence of CMV DNAemia, much more than its effect on HIV transmission, among the breastfed children (table 3). Currently, many studies in developing countries have investigated the role of exclusive breastfeeding while giving HAART to either the mother or the infant. The concept of using HAART to prevent transmission of HIV from the mother to the child via breastfeeding(7;47) is attractive. However, these studies have shown that when the PMTCT fails in these circumstances, the outcomes are generally poor due to drug resistant virus in the infected infants. In most circumstances, the sub-therapeutic level of HAART in breast milk is unable to suppress the HIV viral replication in that compartment and the transmission of these selected strains of HIV results in the infant infected with drug resistant HIV de novo. Our study findings present another aspect which should be noted when the exclusive breastfeeding approach is taken. Current literature indicates that CMV DNAemia is a known contributor to HIV disease progression. The failure of PMTCT in the setting of breastfeeding will undoubtedly result in a high proportion of infants with early HIV and CMV dual infection which favours rapid HIV disease progression. This may in part explain some of the poor outcomes of cases where this PMTCT strategy has failed.

When a new diagnostic assay becomes available, in addition to evaluating its utility, the performance of the existing assay can be similarly examined. CMV DNAemia identifies infants with systemic CMV infection and this can be used as a gold standard for which the sensitivity and specificity of CMV culture could be assessed. The performance of shell vial culture performed on NPA and lower respiratory tract samples (BAL and induced sputum) was compared with CMV DNAemia in infants with pneumonia.

As CMV is commonly shed in the saliva among CMV infected infants, one would expect an NPA from an infant who had detectable CMV in the blood to be culture positive as well. Our data showed that culture of NPA was only able to detect 50% of CMV infections identified by DNAemia. Though culture of NPA samples detected 3 additional CMV infections in non-DNaemic infants, the overall sensitivity of the assay for CMV infection was poor. This low pick up rate could reflect poor sample quality, technical problems with CMV shell vial assay, or lack of viral shedding from the nasopharynx despite CMV infection. Further studies using CMV PCR on respiratory samples are underway to determine the exact sensitivity of the CMV culture in this context. Currently the low yield of CMV viral culture is considered a benefit as it is thought only a high level of viral shedding would be detected by this relatively insensitive method. However, there is no evidence that CMV pneumonia has any association with CMV shedding from the upper respiratory tract. CMV is frequently shed in the oropharynx by healthy individuals and thus culture of CMV from the upper respiratory tract is also likely to yield low specificity for CMV pneumonia. The combination of above factors suggests that CMV culture from NPA may be a fruitless laboratory investigation and the current clinical practice may need to be altered.

The performance for culture from the lower respiratory tract at a glance looks similar to that from upper respiratory tract. Its current clinical use is to detect CMV pneumonia and a culture positive BAL or IS in conjunction with CMV DNAemia is considered diagnostic of CMV pneumonia in an infant with compatible clinical feature(3). This clinical testing strategy is biologically plausible. Systemic CMV activity plus isolation of CMV at the site of disease is a reasonable indicator for CMV pneumonia. However, the sensitivity of culture from the lower respiratory tract is a cause for concern and literature on the sensitivity of culture from BAL are divided(14;24;34;49). Further examination of the data revealed that the culture result of upper and lower respiratory tract had 91% concordance. It is possible that the technique of viral culture may be an insensitive marker of CMV disease. Some studies have suggested that a proportion of CMV pneumonia is a result of immunopathology and therefore isolation of the organism may not represent

the full picture of CMV EOD at the lung(23). From the sampling perspective, retrieving a good sample from the lower respiratory tract is both labour and skill intensive. When all the above factors are taken into consideration, viral culture from BAL alone may not be sufficient to diagnose CMV pneumonia and an adiitional good laboratory marker of CMV EOD is still needed in this clinical context.

While the presence of viraemia may support the diagnosis of CMV EOD in certain contexts(10), in infants it commonly reflects recent primary CMV infection(55) or subclinical CMV reactivation. It is therefore important to quantify the level of viraemia and compare to the level found in asymptomatic infants. Despite the high prevalence of DNAemia found in the HIV uninfected infants with pneumonia, the mean CMV viral load (3.4 ± 0.9 log copies/ml) was comparable to that seen in the asymptomatic infants (3.5 ± 0.9 log copies/ml), and significantly lower than that in HIV infected infants with pneumonia (4.3 ± 1.2 log copies/ml). This is not surprising as CMV is an uncommon cause of pneumonia in immunocompetent infants. The slightly higher CMV viral load seen in the HIV negative infants with pneumonia could reflect CMV reactivation in the face of another serious illness. The significantly higher CMV VL seen in the HIV positive infants, on the other hand, suggests that this marker is able to distinguish between asymptomatic reactivation and CMV EOD. However, these results alone only suggest that significant CMV DNAemia is more likely to be present in an HIV infected infants with pneumonia. Further, the range of CMV VL's for both asymptomatic infants and infants with severe pneumonia were both broad, ranging from the absolute lower detection limit of around 2 log genome copies/ml to >6 log copies/ml. There was also significant overlap in the IQR between the HIV infected asymptomatic infants (3.6 ± 1 log copies/ml) and its pneumonic counterpart (4.3 ± 1.2 log copies/ml). To determine whether CMV VL can accurately differentiate CMV EOD from CMV reactivation, more analyses need to be performed comparing infants with and without laboratory confirmed CMV EOD.

therefore infants with multiple pathogens should not be excluded from the analysis of CMV disease.

We found that the infants with probable CMV related pneumonia had a significantly higher mean CMV viral load (4.56 log copies/ml) compared to the HIV uninfected infants with pneumonia (3.6 logs copies/ml) ($p<0.01$). This mean viral load was also significantly higher than in asymptomatic HIV positive infants ($p<0.01$) and asymptomatic HIV negative infants ($p<0.01$). In clinical practice, CMV PCR is often performed first to rule out CMV disease as this test has a high negative predictive value. We therefore analysed the data further by only comparing DNAemic infants with and without pneumonia (Figure 6). The level of DNAemia was still significantly higher in the DNAemic infants with pneumonia group. This confirms findings from other clinical settings that the quantitative CMV viral load has a role in predicting CMV disease. However, the infants with high CMV VL in the non-CMV pneumonia are a cause for concern. These infants could have CMV related disease in another organ. Unlike in adults, CMV end organ disease such as retinitis and oesophagitis in infants does not present with specific complaint. When these extra-pulmonary EOD present concurrently with pneumonia, the diagnosis may be missed. Thus regardless of whether CMV was cultured in the respiratory tract, presence of high level of CMV DNAemia should alert the clinician to the possibility of CMV EOD.

Determining a threshold DNAemia for CMV disease

ROC curve analysis is a common tool used to evaluate and refine the performance of medical diagnostic assays. A data set with known true positive results (or results using a gold standard assay) is required for this analysis. Using individual values in the data set as the cut-off for the rest of the data set, a list of sensitivities and specificities can be generated for each value. This is plotted on a graph with y axis being sensitivity and x axis 1minus specificity (1- specificity i.e. false positive rate). From this list of sensitivities, one can determine the best cut-off that is able to predict most of the result correctly. Simply put, it is a tool to establish a cut-off that would maximize sensitivity and minimize false positivity.

In the severe pneumonia component of this study, we used a set of clinical and laboratory criteria as the "gold standard" for diagnosing CMV-related pneumonia. The CMV VL was then measured for each of the infants in this cohort with the non-viraemic children's CMV VL set at zero log copies/ml whole blood. The ROC analysis performed from this data indicated that using a cut-off of 4.09 genome log we would be able to produce the best concordance rate with the gold standard, correctly identifying infants with CMV-related pneumonia in 81% of cases.

Importantly, the result of ROC curve analysis would depend on the dataset collected. Just as the disease prevalence of the cohort would influence positive and negative predictive value of an assay, so would characteristics of the cohort examined influence the cut-off determined by the ROC analysis. Various different sets of CMV VL cut- offs have been established by previous studies done in various other clinical contexts. Some of the cut-off's were reviewed by Arav-Boger and Pass(4). Both Lilleri et al (32)and Cope et al (13) used CMV VL of >100,000 on whole blood as a cut-off to predict CMV disease in solid organ transplant recipient whereas Boppana (8) and colleagues found 10,000 genome equivalents/ml to be predictive of symptomatic disease in congenitally and perinatally infected infants. In studies focusing on HIV infected individuals, Brantsaeter et al (10) found that a cut-off of 10,000 copies/ml yielded a positive predictive value of 100%

when compared to post mortem histological findings. Wiselka and colleague(53), on the other hand, found the cut-off of 100 copies/ml have the positive and negative predictive value of 44% and 82% respectively.

It is very difficult to interpret these findings and extrapolate them to our paediatric setting for many reasons. Firstly there is no internationally standardized measurement of CMV genome. The findings of the above studies are all done using different methods. Without a denominator, these assays, many of which are in house, cannot be compared. Secondly the type of blood compartment (plasma, whole blood or peripheral blood leukocytes) tested was also different. The IHMF recommends whole blood as the ideal compartment for the measurement of CMV DNAemia due to its sensitivity and practicality. Perhaps future studies will heed this recommendation and use whole blood as the starting point of DNAemia estimate. Thirdly the targets of PCR and methodology of PCR were also not standardized. Finally, other environmental factors may influence the level of viraemia in different studies. Thus in order to establish a threshold for our local paediatric setting, we can only use local data to guide us. Nontheless, the cut-off determined in the study seems to be in line with the findings of others, namely that a cut-off between 4-5 logs copies/ml whole blood is likely to be useful to predict CMV EOD.

ROC curve analysis can also be used to estimate the overall performance of the assay by looking at the area under the curve (AUC). The AUC represents the assay performance at all cut-off values. The range is between 0.5 and 1. The closer the individual plots are to the y axis, the higher the AUC. The higher the AUC is, the better the assay performance. Our CMV VL had an AUC of 0.86 (95% CI 0.78-0.93) which shows in this clinical setting the assay has a fairly good diagnostic utility.

Unfortunately without autopsy findings and prospective testing, our study was unable to truly delineate CMV related pneumonia from the rest. A prospective clinical study in which infants are routinely biopsied for CMV histology would be required to truly determine the sensitivity and specificity of CMV viral load.

In the absence of such a study, we believe that CMV viral load may play an important role in guiding the clinician to better manage these infants.

Chapter 5 Conclusion

In conclusion, we report the novel finding that asymptomatic CMV viraemia in HIV-exposed infants is common. Forty one percent of asymptomatic and 71% of ill infants with pneumonia were found to have CMV DNAemia. This high prevalence of DNAemia would negatively influence the PPV of qualitative CMV PCR for diagnosing CMV pneumonia. The presence of CMV DNA in the blood of ill infants does not discriminate between infection from disease and should be interpreted with caution. DNAemia, however, may still be a useful screening tool to identify HIV infected infants at risk of CMV disease or accelerated HIV progression and to exclude CMV disease as its NPV is high.

We further noted that CMV DNAemia was much more common in HIV infected infants than HIV-uninfected infants and acutely ill patients (represented by our severe pneumonia study group) were much more likely to have CMV DNAemia than their asymptomatic counterparts. This difference in prevalence may not translate into clinical significance, as quantitative results indicate that only HIV infected infants with pneumonia had a significantly higher CMV viral load in the study groups examined.

From infants with severe pneumonia, our data indicated that breastfeeding is a significantly associated with CMV DNAemia. Breastfeeding in HIV infected infants may thus represent a risk factor for CMV infection and disease.

The evaluation of existing viral culture techniques in infants with severe pneumonia showed that upper and lower respiratory tract samples yielded similar low NPV when compared to DNAemia. The role of CMV culture from respiratory tract in diagnosing CMV pneumonia would be better evaluated in a prospective study but viral culture of NPA is not helpful for detecting CMV pneumonia.

Despite the significantly higher CMV viral load in infants with probable CMV pneumonia, we could not identify a cut-off that produced clear separation between those with CMV disease and those without. The cut-off of 4 log copies/ml of whole blood was able to correctly identify 81% of infants with

suspected CMV pneumonia in our study. This is in line with the threshold found in other international studies. This would be a good starting point for which future clinical studies can assess the relationship between CMV end organ disease and CMV viraemia.

Reference List

1. Adhikari M, Kauchali S, Moodley A. Clinical profile and morbidity pattern of infants born to HIV infected mothers in Durban South Africa. Indian Pediatr 2006;43(9):804-8.

2. Adler SP. Molecular epidemiology of cytomegalovirus: viral transmission among children attending a day care center, their parents, and caretakers. J Pediatr 1988;112(3):366-72.

3. Angelici E, Contini C, Sebastiani G et al. Cytomegalovirus in bronchoalveolar lavage specimens from patients with AIDS: comparison with antigenaemia and viraemia. J Med Microbiol 1996;45(2):149-52.

4. Arav-Boger R, Pass R. Viral load in congenital cytomegalovirus infection. Herpes 2007;14(1):17-22.

5. Arav-Boger R, Pass RF. Diagnosis and management of cytomegalovirus infection in the newborn. Pediatr Ann 2002;31(11):719-25.

6. Barbi M, Binda S, Caroppo S. Diagnosis of congenital CMV infection via dried blood spots. Rev Med Virol 2006;16(6):385-92.

7. Bedri A, Gudetta B, Isehak A et al. Extended-dose nevirapine to 6 weeks of age for infants to prevent HIV transmission via breastfeeding in Ethiopia, India, and Uganda: an analysis of three randomised controlled trials. Lancet 2008;372(9635):300-13.

8. Boppana SB, Fowler KB, Pass RF et al. Congenital cytomegalovirus infection: association between virus burden in infancy and hearing loss. J Pediatr 2005;146(6):817-23.

9. Bowen EF, Sabin CA, Wilson P et al. Cytomegalovirus (CMV) viraemia detected by polymerase chain reaction identifies a group of HIV-positive patients at high risk of CMV disease. AIDS 1997;11(7):889-93.

10. Brantsaeter AB, Holberg-Petersen M, Jeansson S, Goplen AK, Bruun JN. CMV quantitative PCR in the diagnosis of CMV disease in patients with HIV-infection - a retrospective autopsy based study. BMC Infect Dis 2007;7:127.

11. Chandwani S, Kaul A, Bebenroth D et al. Cytomegalovirus infection in human immunodeficiency virus type 1-infected children. Pediatr Infect Dis J 1996;15(4):310-4.

12. Chintu C, Mudenda V, Lucas S et al. Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. Lancet 2002;360(9338):985-90.

13. Cope AV, Sabin C, Burroughs A, Rolles K, Griffiths PD, Emery VC. Interrelationships among quantity of human cytomegalovirus (HCMV) DNA in blood, donor-recipient

serostatus, and administration of methylprednisolone as risk factors for HCMV disease following liver transplantation. J Infect Dis 1997;176(6):1484-90.

14. Costa C, Libertucci D, Solidoro P et al. Rapid shell vial culture for the detection of respiratory viruses from bronchoalveolar lavage in immunocompromised patients. Panminerva Med 2007;49(1):1-6.

15. Craig JM, Macauley JC, Weller TH, Wirth P. Isolation of intranuclear inclusion producing agents from infants with illnesses resembling cytomegalic inclusion disease. Proc Soc Exp Biol Med 1957;94(1):4-12.

16. Doyle M, Atkins JT, Rivera-Matos IR. Congenital cytomegalovirus infection in infants infected with human immunodeficiency virus type 1. Pediatr Infect Dis J 1996;15(12):1102-6.

17. Emery VC, Sabin CA, Cope AV, Gor D, Hassan-Walker AF, Griffiths PD. Application of viral-load kinetics to identify patients who develop cytomegalovirus disease after transplantation. Lancet 2000;355(9220):2032-6.

18. Fowler KB, Stagno S, Pass RF, Britt WJ, Boll TJ, Alford CA. The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. N Engl J Med 1992;326(10):663-7.

19. Frenkel LD, Gaur S, Tsolia M, Scudder R, Howell R, Kesarwala H. Cytomegalovirus infection in children with AIDS. Rev Infect Dis 1990;12 Suppl 7:S820-S826.

20. Gor D, Sabin C, Prentice HG et al. Longitudinal fluctuations in cytomegalovirus load in bone marrow transplant patients: relationship between peak virus load, donor/recipient serostatus, acute GVHD and CMV disease. Bone Marrow Transplant 1998;21(6):597-605.

21. Graham SM. Impact of HIV on childhood respiratory illness: differences between developing and developed countries. Pediatr Pulmonol 2003;36(6):462-8.

22. Graham SM, Coulter JB, Gilks CF. Pulmonary disease in HIV-infected African children. Int J Tuberc Lung Dis 2001;5(1):12-23.

23. Grundy JE. Virologic and pathogenetic aspects of cytomegalovirus infection. Rev Infect Dis 1990;12 Suppl 7:S711-S719.

24. Hansen KK, Vestbo J, Benfield T, Lundgren JD, Mathiesen LR. Rapid detection of cytomegalovirus in bronchoalveolar lavage fluid and serum samples by polymerase chain reaction: correlation of virus isolation and clinical outcome for patients with human immunodeficiency virus infection. Clin Infect Dis 1997;24(5):878-83.

25. Ishigaki S, Takeda M, Kura T et al. Cytomegalovirus DNA in the sera of patients with cytomegalovirus pneumonia. Br J Haematol 1991;79(2):198-204.

26. Jeena PM. Can the burden of pneumonia among HIV-infected children be reduced? Bull World Health Organ 2008;86(5):323-3A.

27. Jeena PM, Coovadia HM, Chrystal V. Pneumocystis carinii and cytomegalovirus infections in severely ill, HIV-infected African infants. Ann Trop Paediatr 1996;16(4):361-8.

28. Jeffries DJ. The spectrum of cytomegalovirus infection and its management. J Antimicrob Chemother 1989;23 Suppl E:1-10.

29. Kaye S, Miles D, Antoine P et al. Virological and immunological correlates of mother-to-child transmission of cytomegalovirus in The Gambia. J Infect Dis 2008;197(9):1307-14.

30. Keen GA. Human cytomegalovirus infection. S Afr Med J 1985;68(3):159-61.

31. Kovacs A, Schluchter M, Easley K et al. Cytomegalovirus infection and HIV-1 disease progression in infants born to HIV-1-infected women. Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted HIV Infection Study Group. N Engl J Med 1999;341(2):77-84.

32. Lilleri D, Baldanti F, Gatti M et al. Clinically-based determination of safe DNAemia cutoff levels for preemptive therapy or human cytomegalovirus infections in solid organ and hematopoietic stem cell transplant recipients. J Med Virol 2004;73(3):412-8.

33. Limaye AP, Kirby KA, Rubenfeld GD et al. Cytomegalovirus reactivation in critically ill immunocompetent patients. JAMA 2008;300(4):413-22.

34. Mann M, Shelhamer JH, Masur H et al. Lack of clinical utility of bronchoalveolar lavage cultures for cytomegalovirus in HIV infection. Am J Respir Crit Care Med 1997;155(5):1723-8.

35. Mattes FM, McLaughlin JE, Emery VC, Clark DA, Griffiths PD. Histopathological detection of owl's eye inclusions is still specific for cytomegalovirus in the era of human herpesviruses 6 and 7. J Clin Pathol 2000;53(8):612-4.

36. Mofenson LM, Oleske J, Serchuck L, Van Dyke R, Wilfert C. Treating opportunistic infections among HIV-exposed and infected children: recommendations from CDC, the National Institutes of Health, and the Infectious Diseases Society of America. Clin Infect Dis 2005;40 Suppl 1:S1-84.

37. Mussi-Pinhata MM, Yamamoto AY, Figueiredo LT, Cervi MC, Duarte G. Congenital and perinatal cytomegalovirus infection in infants born to mothers infected with human immunodeficiency virus. J Pediatr 1998;132(2):285-90.

38. Nigro G, Krzysztofiak A, Gattinara GC et al. Rapid progression of HIV disease in children with cytomegalovirus DNAemia. AIDS 1996;10(10):1127-33.

39. Peckham CS, Chin KS, Coleman JC, Henderson K, Hurley R, Preece PM. Cytomegalovirus infection in pregnancy: preliminary findings from a prospective study. Lancet 1983;1(8338):1352-5.

40. Razonable RR, Emery VC. Management of CMV infection and disease in transplant patients. 27-29 February 2004. Herpes 2004;11(3):77-86.

41. Rennert WP, Kilner D, Hale M, Stevens G, Stevens W, Crewe-Brown H. Tuberculosis in children dying with HIV-related lung disease: clinical-pathological correlations. Int J Tuberc Lung Dis 2002;6(9):806-13.

42. Rouet F, Sakarovitch C, Msellati P et al. Pediatric viral human immunodeficiency virus type 1 RNA levels, timing of infection, and disease progression in African HIV-1-infected children. Pediatrics 2003;112(4):e289.

43. Rowe WP, Hartley JW, Waterman S, Turner HC, Huebner RJ. Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids. Proc Soc Exp Biol Med 1956;92(2):418-24.

44. Rubin RH. Impact of cytomegalovirus infection on organ transplant recipients. Rev Infect Dis 1990;12 Suppl 7:S754-S766.

45. Salomon N, Gomez T, Perlman DC, Laya L, Eber C, Mildvan D. Clinical features and outcomes of HIV-related cytomegalovirus pneumonia. AIDS 1997;11(3):319-24.

46. Smith MG. Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus (SGV) disease. Proc Soc Exp Biol Med 1956;92(2):424-30.

47. Thior I, Lockman S, Smeaton LM et al. Breastfeeding plus infant zidovudine prophylaxis for 6 months vs formula feeding plus infant zidovudine for 1 month to reduce mother-to-child HIV transmission in Botswana: a randomized trial: the Mashi Study. JAMA 2006;296(7):794-805.

48. Torres-Madriz G, Boucher HW. Immunocompromised hosts: perspectives in the treatment and prophylaxis of cytomegalovirus disease in solid-organ transplant recipients. Clin Infect Dis 2008;47(5):702-11.

49. Uberti-Foppa C, Lillo F, Terreni MR et al. Cytomegalovirus pneumonia in AIDS patients: value of cytomegalovirus culture from BAL fluid and correlation with lung disease. Chest 1998;113(4):919-23.

50. Waxman AB, Goldie SJ, Brett-Smith H, Matthay RA. Cytomegalovirus as a primary pulmonary pathogen in AIDS. Chest 1997;111(1):128-34.

51. Weinberg A, Duarte MI. Respiratory complications in Brazilian patients infected with human immunodeficiency virus. Rev Inst Med Trop Sao Paulo 1993;35(2):129-39.

52. Williams AJ, Duong T, McNally LM et al. Pneumocystis carinii pneumonia and cytomegalovirus infection in children with vertically acquired HIV infection. AIDS 2001;15(3):335-9.

53. Wiselka MJ, Nicholson KG, Rowley S, Bibby K. Cytomegalovirus viraemia has poor predictive value for the development of cytomegalovirus disease in patients with advanced HIV-infection. J Infect 1999;39(3):187-92.

54. Zar HJ, Dechaboon A, Hanslo D, Apolles P, Magnus KG, Hussey G. Pneumocystis carinii pneumonia in South African children infected with human immunodeficiency virus. Pediatr Infect Dis J 2000;19(7):603-7.

Comparison between CMV related pneumonia and non-CMV related pneumonia among children with severe pneumonia

In Sun-Saharan Africa where CMV prevalence is high among children, HIV and pneumonia disease burdens are both high. 80% of HIV infected infants develop respiratory tract illness during their course of disease. HIV infected African infants admitted with severe pneumonia is associated with a case fatality rate of 20% to 34%; three- to six-times higher than children who are not infected with HIV(26). Although the aetiology of this severe pneumonia is polymicrobial, CMV has been found more frequently in HIV infected infants in studies examining cause of the severe pneumonia(12;22;27;54) and the outcomes of CMV pneumonia among HIV infected individuals are poor(41;50;51). This is in stark contrast of the pre-HAART era of first world countries where CMV plays little role in the respiratory illness of HIV infected infants(21;54). Despite this significant health problem unique to developing countries, there are very few studies that address the diagnosis of CMV associated pneumonia. As a result clearly defined CMV pneumonia among HIV infected infants is under represented in the literature.

To define the CMV DNA level associated with CMV disease, we chose to study infants with CMV related pneumonia, a common clinical manifestation of CMV disease in our setting. The parameters used in our case definition were largely based on criteria commonly used to define CMV pneumonia in adult HIV infection and transplant recipients (20;45). We deviated from the original definition in that we did not exclude individuals from whom another microbe had been isolated. The main reason for this was that many studies have shown that HIV infected infants are prone to respiratory tract infection caused by more than one pathogen, including bacteria, viruses and fungi. In each of these situations, CMV may play an important role in exacerbating the respiratory tract infection. In most cases of CMV infected children with severe pneumonia, there is an additional pathogen present. Previous autopsy studies have identified CMV as an important contributor to pathology and

55. Ziemann M, Krueger S, Maier AB, Unmack A, Goerg S, Hennig H. High prevalence of cytomegalovirus DNA in plasma samples of blood donors in connection with seroconversion. Transfusion 2007;47(11):1972-83.